



## Environmental and genetic factors influence the vitamin D content of cows' milk

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1 **Environmental and genetic factors influence the vitamin D content of cows' milk**

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13

**Abstract**

Vitamin D is obtained by cattle from the diet and from skin production via ultraviolet (UV)-B exposure from sunlight. The vitamin D status of the cow impacts the vitamin D content of the milk produced, much like human breast milk, with seasonal variation in the vitamin D content of milk well documented. Factors such as changes in husbandry practices therefore have the potential to impact the vitamin D content of milk. For example, a shift to year-round housing from traditional practises of cattle being out to graze during the summer months and housed during the winter only, minimises exposure to the sun and has been shown to negatively influence the vitamin D content of the milk produced. Other practices such as changing dietary sources of vitamin D may also influence the vitamin D content of milk, and evidence exists to suggest genetic factors such as breed can cause variation in the concentrations of vitamin D in the milk produced. This review aims to provide an overview of the current understanding of how genetic and environmental factors influence the vitamin D content of the milk produced by dairy cattle. A number of environmental and genetic factors have previously been identified as having influence on the nutritional content of the milk produced. This review highlights a need for further research to fully elucidate how farmers could manipulate the factors identified to their advantage with respect to increasing the vitamin D content of milk and standardising it across the year.

## Introduction

Cattle require vitamin D to aid the excretion of calcium from the kidneys and in the reabsorption of calcium from the bones, maintaining calcium homeostasis<sup>(1)</sup>. Vitamin D is also important in preventing the development of hypocalcaemia<sup>(2)</sup> and milk fever which is a debilitating disorder typically seen close to calving, characterised by decreased blood calcium concentrations, and in severe cases can result in fatalities<sup>(3)</sup>.

In a similar manner to humans, cattle can obtain vitamin D through both endogenous, or dermal synthesis, as well as dietary sources. Only vitamin D<sub>3</sub> (cholecalciferol) is produced through dermal synthesis following exposure to ultraviolet (UV)-B emitted from the sun<sup>(4)</sup>. Dietary sources, however, can provide both vitamin D<sub>3</sub> and vitamin D<sub>2</sub> (ergocalciferol). Vitamin D<sub>2</sub> is typically obtained naturally through the ingestion of fungi growing among the vegetation cattle consume<sup>(5)</sup>, and dietary vitamin D<sub>3</sub> is provided through synthetic additives in the feed concentrates<sup>(6)</sup>, usually in regulated quantities (per kg/day). Therefore, differences in husbandry practices can cause an inherent variation in the vitamin D content of the milk produced between different farms and throughout the year (e.g. housed vs. grazing on pasture, and grass vs. concentrate feed). The amount of vitamin D consumed or synthesised by cattle impacts the vitamin D status of the animal, and much like human breast milk, the vitamin D status of the cow subsequently impacts the vitamin D content of the milk produced<sup>(7, 8, 9)</sup>.

Cows' milk provides many nutrients in the human diet (e.g. protein, calcium, riboflavin, vitamin B12, potassium, iodine and phosphorus) and has been associated with a number of health benefits<sup>(10)</sup>. In the face of limited dietary sources of vitamin D, dairy products remain an important contribution to adults' overall vitamin D intake<sup>(11)</sup>, with several countries across the globe implementing a mandatory or voluntary fortification policy for fluid milk to improve the vitamin D content of the milk on sale<sup>(12, 13, 14, 15)</sup>.

The aims of this review were to provide an overview of (1) the genetic and the environmental factors that influence the vitamin D status of dairy cattle, and; (2) how these factors influence the vitamin D content of the milk produced.

## Environmental Factors

### *Seasonal changes in vitamin D content*

Seasonal variations in vitamin D content of milk are well documented, with concentrations found to be higher in the summer months than in the winter, most likely owing to differences in both husbandry and feeding practices between the seasons. Reports dating back as far as the 1920's demonstrated that a single cow pasture-fed between May and July had a higher 'anti-rachitic' (vitamin D) content than the milk produced when the same cow was fed in-house and kept in the dark<sup>(16)</sup>. The same cow was then involved in another study, which collected milk samples for 18 months. In support of the initial findings, a 2-3-fold increase in the vitamin D content of the milk produced was observed when the cow was out to pasture, compared to the milk produced when the cow was housed in a dark stall<sup>(17)</sup>. Evidence suggests that this seasonal variation is the result of insufficient stores of vitamin D in the liver and fat tissue for mobilisation in times when dietary intake of the vitamin is low<sup>(18)</sup>. Many subsequent studies have confirmed the seasonal variation of the vitamin D content of milk (approximate differences ranging between 0.004 – 0.0014µg per gram of fat) across different countries and breeds of cattle (Table 1)<sup>(24, 25, 26, 27)</sup>. Although seasonal variation in vitamin D content is widely reported in the literature, units of measurement are inconsistent which makes it difficult to compare between studies. In the previous edition of the UK Food Composition Tables, no seasonal variation in the vitamin D content of milk was noted for whole, semi-skimmed and skimmed milk, but was observed in the whole milk samples from the Channel Islands, where mean concentrations for summer and winter were 0.04µg/100g and 0.03µg/100g, respectively<sup>(23)</sup>. In the most recent edition a lack of seasonal variation is still apparent, with vitamin D only quantified for Channel Island whole milk, listed as 0.01µg/100g and trace for whole, semi-skimmed, skimmed and one-percent milks<sup>(24)</sup>.

While the seasonal variation in the vitamin D content of milk is established, not all studies or databases, such as the recent editions of the UK Food Composition Tables, report such variations, and a more comprehensive update of vitamin D in milk across the UK and Ireland is warranted.

### ***UVB Exposure***

In a study by Hymøller, cows from two organic dairy farms in Denmark were selected to determine the effect of sunlight on vitamin D status in March and April, and on each farm, cattle were allocated based on milk yield, parity and lactation stage to have daily outdoor access (from February to April) or to be confined indoors for the duration of the study (November – April)<sup>(25)</sup>. Results from Farm 1 found no significant effect of treatment allocation on plasma 25(OH)D<sub>3</sub> concentration in March ( $P=0.350$ ) or April ( $P=0.060$ ), with mean plasma 25(OH)D<sub>3</sub> concentrations of 7.84ng/ml and

5.85ng/ml for the outdoor and indoor groups, respectively<sup>(25)</sup>. On Farm 2, the outdoor group had a significantly higher 25(OH)D<sub>3</sub> concentration in March, compared to the indoor group (5.71ng/ml vs. 3.36ng/ml;  $P<0.05$ ), but the same difference was not reported in April ( $P=0.100$ )<sup>(25)</sup>. Hymøller and colleagues<sup>(25)</sup> concluded that the assumption was that supplemental vitamin D<sub>3</sub> may still be required in the spring as a means to maintain a healthy vitamin D status.

In the field of bio-fortification/bio-addition, a recent Danish study<sup>(9)</sup> investigated the potential impact of supplemental UVB light on vitamin D<sub>3</sub> synthesis in 16 housed Holstein cattle, a common dairy breed, which had been severely depleted of their vitamin D stores. The cows were randomised to receive artificial UVB light 30, 90 or 120 minutes daily for 24 days, or 60 minutes for 73 days; the length of UVB exposure was designed to be equivalent to 1, 2, 3 and 4 hours of sunlight at pasture at 56°N respectively<sup>(9)</sup>. After 24 days, exposure to supplemental UVB light significantly increased the vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> concentrations in the milk in a dose-dependent manner over 30, 90 and 120 minutes<sup>(9)</sup>. In the cattle allocated to receive 60 minutes daily, a significant increase ( $P=0.029$ ) in the vitamin D<sub>3</sub> (but not the 25(OH)D<sub>3</sub>) concentration of the milk produced between day 0 and 24 was noted, but this did not increase further up to day 73 ( $P=0.400$ )<sup>(9)</sup>.

This important preliminary evidence, albeit from a limited number of studies, suggests that vitamin D bio-fortification of cow's milk does, at least in theory seem probable. Future studies therefore should investigate this novel on-farm method as a means of minimising the seasonal variation in cow's vitamin D status and the milk produced.

## ***Diet***

The seasonal changes in the vitamin D content of milk, have long been associated with the change in UV intensity and a reduction in the time spent outdoors, rather than as a result of the change in feed<sup>(22, 26)</sup>. That being stated, in the UK cattle are solely reliant on dietary vitamin D during the winter, obtained through grass stores (hay, silage or haylage) or feed concentrates. Prior to 2010, both vitamin D<sub>2</sub> and D<sub>3</sub> were authorised by the European Commission as sources of vitamin D which could be added to feeds intended for cattle; however, in November 2010 no submission was made for the re-authorisation of a vitamin D<sub>2</sub> dossier, and as a result cattle can now only obtain vitamin D<sub>2</sub> from the consumption of fungi growing among the vegetation (fresh grass, hay, silage or haylage) used as roughage in the diet<sup>(5)</sup> and not from concentrates. Within the EU, vitamin D<sub>3</sub> is now the only authorised source of supplemental vitamin D for cattle<sup>(27)</sup>, with maximum permitted levels set at 4,000IU (100µg) per kg of feed<sup>(28)</sup>.

126 Although cattle are reliant on dietary vitamin D during the winter months, it has been suggested that  
 127 fat soluble vitamins from such dietary sources are destroyed once they enter the rumen, owing to the  
 128 fermentative environment<sup>(29, 30)</sup>. Research using a fistula model was designed to test this hypothesis  
 129 in vitamin D<sup>(4)</sup>. A maximum of 15kg of ruminal contents were removed and mixed with a vitamin D<sub>2</sub>  
 130 and D<sub>3</sub> (both 250mg) and vitamin E pre-mix<sup>(4)</sup>. The contents were then returned to the rumen; ruminal  
 131 and blood samples were then collected over the subsequent 30 hour period<sup>(4)</sup>. Once collected, ruminal  
 132 samples were freeze dried (*in vivo* samples), additional ruminal samples were collected at the 1 hour  
 133 time-point, and stored in plastic bottles which were then placed in a water bath (37°C) (*in vitro*  
 134 samples). Samples were then removed from the *in vitro* over the 30 hour period and freeze dried for  
 135 analysis. The concentrations of both vitamin D<sub>2</sub> and D<sub>3</sub> declined over the study period in the *in vivo*  
 136 samples, with concentrations remaining stable in the *in vitro* samples, suggesting no degradation in  
 137 the intact ruminal sample<sup>(4)</sup>. Results showed that the plasma concentrations of both vitamin D<sub>2</sub> and  
 138 vitamin D<sub>3</sub> increased over the first few hours, from levels below the limit of detection, and reached a  
 139 maximum concentration after 24 hours ( $99 \pm 15\text{ng/ml}$  and  $163 \pm 16\text{ng/ml}$ , respectively), with vitamin  
 140 D<sub>3</sub> concentrations significantly higher than those for vitamin D<sub>2</sub><sup>(4)</sup>. It has previously been  
 141 hypothesised that vitamin D degradation in the rumen may be a natural protective detoxification  
 142 process when large quantities of the vitamin are consumed<sup>(31)</sup>, and this may also be a possible reason  
 143 for the rapid conversion to 25(OH)D observed by Hymøller and Jensen<sup>(4)</sup>.

144 Previously the potential of intravenous supplements to improve the vitamin D status of the cow and  
 145 the milk produced have also been considered. Thompson and Hidiroglou<sup>(32)</sup> orally administered  
 146 1,000,000 IU (25,000µg) of vitamin D<sub>2</sub> and 1,000,000 IU (25,000µg) of vitamin D<sub>3</sub> mixed in corn oil  
 147 to 2 dairy cows, collecting milk and blood samples for 10 days after. The results showed that the  
 148 maximum plasma vitamin D concentrations were observed after 2-3 days, with maximum  
 149 concentrations in the milk 1-3 days after<sup>(32)</sup>. At the same time 12 additional cows were allocated to  
 150 be orally or intravenously administered with vitamin D<sub>3</sub> in doses of 5,000,000 IU (125,000µg) or  
 151 10,000,000 IU (250,000µg). Increases in the vitamin D content of the milk produced varied between  
 152 animal, with maximum levels reached between 3-7 days for the oral doses and up to 10 days for the  
 153 intravenous doses, with maximum observed ranges between 8 IU (0.2µg) – 92 IU (2.3µg) per  
 154 100ml<sup>(32)</sup>. It is important to interpret these results with caution as the doses administered in this trial  
 155 are extreme and would not be feasible to incorporate into the daily management of a dairy herd.  
 156 Furthermore, little is also known on the safety, efficacy and longer-term effects of prolonged usage  
 157 ‘mega-doses’, other than the data available for acute doses used in the treatment of milk fever<sup>(33, 34)</sup>.

158 A research team led by Hollis collected milk samples from two groups of cows [4,000 IU (100µg)  
 159 vs. 40,000 IU (1,000µg) daily], and found concentrations of vitamin D, 25(OH)D and 1,25-

160 dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) in the milk to be greater in those cattle receiving a higher daily  
 161 dose of vitamin<sup>(8)</sup>. Similar results were noted for 24,25-dihydroxyvitamin D (24,25(OH)<sub>2</sub>D) and  
 162 25,26-dihydroxyvitamin D (25,26(OH)<sub>2</sub>D)<sup>(8)</sup>. This research indicates that the intake of sufficient  
 163 quantities of dietary vitamin D is enough to increase the vitamin D content of the milk produced.

164 A cross-over study randomised 14 Danish Holstein cows based on parity and milk yield to receive a  
 165 one-off 250mg dose of vitamin D<sub>2</sub>, followed by the same dose of vitamin D<sub>3</sub> in capsule-form, or vice  
 166 versa<sup>(6)</sup>. Plasma samples were obtained and area under the curve was used to determine the impact of  
 167 the two different doses on plasma 25(OH)D status. Results found that the concentrations of plasma  
 168 25(OH)D<sub>2</sub> when D<sub>2</sub> was administered first was less than half that of 25(OH)D<sub>3</sub> when the vitamin D<sub>3</sub>  
 169 dose was given first ( $P \leq 0.001$ )<sup>(6)</sup>, suggesting that vitamin D<sub>2</sub> may impair the utilisation of vitamin  
 170 D<sub>3</sub>.

171 McDermott and colleagues assigned 20 Holstein cows to receive 0 IU, 10,000 IU (250µg), 50,000 IU  
 172 (1,250µg), or 250,000 IU (6,250µg) of vitamin D<sub>3</sub> daily, for 14-weeks starting at 2 weeks pre-  
 173 partum<sup>(35)</sup>. Vitamin D<sub>3</sub> concentrations in the colostrum were significantly higher ( $P < 0.05$ ) in cows  
 174 receiving 250,000 IU/day compared to the other groups, although this dropped during the transition  
 175 to normal milk from colostrum, around 1 week post-partum. At the end of the study the vitamin D<sub>3</sub>  
 176 content of the milk was approximately 0.075ng/ml, 0.16ng/ml, 20ng/l and 22ng/ml for 0, 10,000,  
 177 50,000 and 250,000 IU respectively<sup>(35)</sup>. A mean concentration of 0.15ng/ml for 25(OH)D<sub>3</sub> was  
 178 observed in normal milk<sup>(35)</sup>.

179 The need to supplement cattle over the summer months with vitamin D<sub>3</sub> was investigated in Swedish  
 180 Holsteins, assigned to receive a mineral feed containing vitamin D<sub>3</sub> concentrations in accordance to  
 181 Swedish recommendations (control) or the same feed providing approximately 20,000 IU (500µg) of  
 182 vitamin D<sub>3</sub> daily<sup>(2)</sup>. Plasma samples collected over the 2-year period showed a significant effect of  
 183 treatment on the cattle's circulating 25(OH)D<sub>3</sub> concentrations compared to control ( $P \leq 0.001$ ) and  
 184 moreover, the 25(OH)D<sub>3</sub> concentrations in the both the supplemented and unsupplemented cows  
 185 increased when the cattle were out at pasture over the summer months<sup>(2)</sup>. The authors concluded that  
 186 cattle obtain adequate vitamin D<sub>3</sub> from dermal synthesis over the summer, but that stores were not  
 187 adequate to maintain status and they had to rely on supplemental vitamin D over the winter<sup>(2)</sup>.

188

189 Overall the results of the above studies provide evidence to suggest that dietary vitamin D<sub>3</sub> is adequate  
 190 to improve the vitamin D content of the milk produced and to help maintain status in times where  
 191 dermal synthesis is not feasible, despite the fermentative environment of the rumen. These findings



are of particular importance in relation to recent changes in husbandry practises, which has seen a growing shift to the year round housing of cattle.

## **Genetic Factors**

### ***Breed***

The variation in the vitamin D content of milk produced by different cattle breeds is supported by evidence conducted across the world (Table 2). The Holstein-Friesian cross has become the most common breed of dairy cow, used for milk production across the world, owing to the high production rates<sup>(36)</sup> and also remains a popular choice within the majority of British herds<sup>(37)</sup>.

The average vitamin D content of milk produced in the UK is currently documented as ‘trace’ for whole, semi-skimmed and skimmed milk, albeit breed is not specified, with the exception of whole milk from the Channels Islands where Jersey cows are the dominant breed (0.1µg/100g)<sup>(24)</sup>. The differences in vitamin D reported in the current Food Composition Tables support the results of Wallis<sup>(38)</sup> who compared the vitamin D content of the milk from Holsteins and Jerseys in the 1940’s. Results from this early work showed that although Holsteins produced vastly greater quantities of milk, the vitamin D content of the Jersey cows was on average 3-fold higher owing to higher butterfat concentrations<sup>(38)</sup>. Bechtel and Hoppert noted that not only was the vitamin D content of the milk higher in the summer months but also that the milk fat produced from the Guernsey cattle was higher than the milk of the Holstein cattle<sup>(39)</sup>. A British study involving three cattle breeds (Friesian, Jersey and Ayrshire) commonly milked in the UK observed differences across the three breeds in summer milk, with little difference apparent in winter milk<sup>(19)</sup>. In Portugal, two studies have noted a higher vitamin D content of milk from indigenous dairy breeds (Barrosã and Minhota) when compared that from with Friesians and Holstein-Friesians<sup>(36, 40)</sup>.

### ***Hair coverage and dominant colour***

To determine if cattle could synthesis vitamin D<sub>3</sub> regardless of hair coverage, Hymøller and Jensen<sup>(41)</sup> designed a study involving 16 Danish Holstein cattle, which had been depleted of their vitamin D stores, and randomised based on parity and milk yield to one of four groups. The treatment groups consisted of different levels of body coverage with a fabric which prevented vitamin D synthesis for 28 days; a horse blanket: an udder cover: a horse blanket and an udder cover: no coverage<sup>(41)</sup>. The cattle were on pasture for 5 hours each day and inside for the remainder of the day, and were fed a

223 vitamin D<sub>3</sub> free diet throughout the study<sup>(41)</sup>. Mean plasma 25(OH)D<sub>3</sub> concentrations increased from  
 224  $2.8 \pm 0.2$  ng/ml at baseline in all treatment groups, in a dose-dependent manner with the increasing  
 225 level of body coverage<sup>(41)</sup> (Table 3).

226 More recently Hymøller & Jensen<sup>(42)</sup> randomised 20 Danish Holstein heifers based on milk yield and  
 227 dominant hair colour (black or white) to five different groups, allocated to an increasing length of  
 228 time on pasture per day (0, 15, 30, 75, 150 or 300 minutes)<sup>(42)</sup>. At baseline, the mean plasma  
 229 25(OH)D<sub>3</sub> concentration for all the heifers was  $44.9 \pm 2.4$  nmol/l. Over 28 days, the cattle on pasture  
 230 for 15, 30 or 75 minutes were unable to maintain their 25(OH)D<sub>3</sub> concentrations from that at baseline.  
 231 A significant increase in mean 25(OH)D<sub>3</sub> concentration was observed however in those outside on  
 232 pasture for 150 or 300 minutes<sup>(42)</sup>. In addition, they found that the dominant coat colour (black or  
 233 white) had no significant effect on the plasma concentrations of 25(OH)D<sub>3</sub>, illustrating that prominent  
 234 coat colour does not influence the dermal synthesis of vitamin D<sub>3</sub> in such cattle<sup>(42)</sup> (Table 3).

235 The results of these two unique studies eloquently demonstrate that cattle can synthesis vitamin D<sub>3</sub>  
 236 through all areas of their skin and not just in the udders or muzzle, where hair coverage is scarce. The  
 237 work by Hymøller and colleagues also illustrates that, unlike humans, pigmentation has no effect on  
 238 the synthesis of vitamin D<sub>3</sub> following UVB exposure<sup>(43)</sup>. Further work in other cattle breeds is  
 239 required to further investigate the variance in vitamin D levels in the milk produced. In addition it  
 240 may be beneficial to further explore the research by Hymøller & Jensen in other breeds to determine  
 241 other factors that may prevent the dermal synthesis of vitamin D, such as longed haired cattle breeds.

242

## 243 **Other Factors**

### 244 *Age*

245 A German two series study investigated the impact age has on the metabolism of 25(OH)D<sub>3</sub><sup>(44)</sup>. In  
 246 the first series, 14 multiparous cows were supplemented orally with 3mg of 25(OH)D<sub>3</sub> daily from  
 247 270<sup>th</sup> day of gestation until parturition, with blood samples collected every other day<sup>(44)</sup>. Ninety cows  
 248 were allocated in the second series to receive 0, 4, 6 mg of 25(OH)D<sub>3</sub> daily through mineral feed  
 249 additives for the last 8-10 days of gestation, with blood samples also taken every other day until  
 250 parturition, and at several intervals thereafter<sup>(44)</sup>. Calculated slopes found the difference in 25(OH)D<sub>3</sub>  
 251 between cattle in their 2<sup>nd</sup> and 3<sup>rd</sup> lactation to be significantly higher in the 2<sup>nd</sup> lactation ( $P < 0.001$ ),  
 252 suggesting that younger cattle are more efficient at absorbing 25(OH)D<sub>3</sub> or that in older cattle the rate  
 253 of 25(OH)D<sub>3</sub> elimination is faster, with 1,25(OH)<sub>2</sub>D<sub>3</sub> increased in cattle in third lactation or higher<sup>(44)</sup>.

254

### *Stage of lactation*

A Japanese study collected milk samples from three Holstein cows at stage points post-partum: 1 day after, colostrum; 2-4 days after, early milk and 15 days after, later milk)<sup>(45)</sup>. Similar concentrations of vitamin D were recorded across the three points for two of the cows [33.2 IU/l (0.83µg/l); 30.9 IU/l (0.77µg/l); 35.6 IU/l (0.89µg/l), respectively and 47.0 IU/l (1.18µg/l); 47.0 IU/l (1.18µg/l); 55.7 IU/l (1.39µg/l), respectively], with no trend noted in the third [77.0 IU/l (1.93µg/l), 88.9 IU/l (2.22µg/l) and 47.4 IU/l (1.19µg/l)]<sup>(45)</sup>.

Further work required to fully elucidate the impact of age and lactation on the vitamin D content of milk, as this has previously been established for other nutrients such as fatty acids<sup>(46, 47)</sup>, this is of importance as the cattle milked on a farm will be at various stages of lactation depending on calving dates and parity.

### **Conclusion**

The present review has identified a number of environmental and genetic factors which can influence both the vitamin D status of cattle and the vitamin D content of the milk produced. It is noteworthy, however, that most of the research investigating the factors influencing the composition of cows' milk are, more often than not, concerned only with the macronutrient (namely protein and fat content). Much of the research available with regards to the vitamin D content of cow's milk is in relation to the prevention and treatment of hypocalcaemia and milk fever in dairy herds. Of particular importance to the dairy industry, this review of the literature indicates that further research is needed to fully elucidate how farmers could manipulate the various factors identified to their advantage with respect to increasing the vitamin D content of milk, and standardising it across the year. Notwithstanding the clear and established health benefits for the animal associated with an improved vitamin D status, this approach potentially could also provide a premium product with an improved vitamin D content for the eventual benefit of the consumer.

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287

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289 A.M.F., S.S. and L.K.P reviewed and approved the final manuscript.

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## References

1. Horst RL, Goff JP, Reinhardt TA (1994) Symposium: Calcium metabolism and utilization. Calcium and vitamin D metabolism in the dairy cow. *J Dairy Sci* **77**, 1936–1951.
2. Hymøller L, Jensen SK, Lindqvist H *et al.* (2009) Supplementing dairy steers and organically managed dairy cows with synthetic vitamin D<sub>3</sub> is unnecessary at pasture during exposure to summer sunlight. *J Dairy Res* **76**, 372–378.
3. Goff JP, Liesegang A, Horst RL (2014) Diet-induced pseudohypoparathyroidism: a hypocalcemia and milk fever risk factor. *J Dairy Sci* **97**, 1520-1528.
4. Hymøller L & Jensen SK (2010) Stability in the rumen and effect on plasma status of single oral doses of vitamin D and vitamin E in high-yielding dairy cows. *J Dairy Sci* **93**, 5748–5757.
5. Richardson MD & Logendra S (1997) Ergosterol as an indicator of endophyte biomass in grass seeds. *J Agric Food Chem* **45**, 3903–3907.
6. Hymøller L & Jensen SK (2011) Vitamin D<sub>2</sub> impairs utilization of vitamin D<sub>3</sub> in high-yielding dairy cows in a cross-over supplementation regimen. *J Dairy Sci* **94**, 3462–3466.
7. Light RF, Wilson LT, Frey CN (1934) Vitamin D in the blood and milk of cows fed irradiated yeast. *J Nutr* **8**, 105-111.
8. Hollis BW, Roos BA, Draper HH *et al.* (1981) Vitamin D and its metabolites in human and bovine milk. *J Nutr* **111**, 1240-1248.
9. Jakobsen J, Jensen SK, Hymøller L *et al.* (2015) Short communication: Artificial ultraviolet B light exposure increases vitamin D levels in cow plasma and milk. *J Dairy Sci* **98**, 1-7.
10. Food and Agriculture Organization (FAO) (2013) Milk and dairy products in human nutrition. FAO, Rome.
11. Nutritional Diet and Nutrition Survey (2014) Results from Years 1,2,3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/2012). Public Health England.
12. Laaksi IT, Ruohola JS, Ylikomi TJ *et al.* (2006) Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. *Eur J Clin Nutr* **60**, 1035-1038.

13. Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D and Calcium (2011) Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC): [National Academies Press \(US\)](#).
14. Calvo MS & Whiting SJ (2013) Survey of current vitamin D food fortification practices in the United States and Canada. *J Steroid Biochem Mol Biol* **136**, 211-213.
15. Lamberg-Allardt C, Brustad M, Meyer HE *et al.* (2013) Vitamin D – a systematic literature review for the 5th edition of the Nordic Nutrition Recommendations. *Food Nutr Res* **57**, 1-31.
16. Luce EM (1924) The influence of diet and sunlight upon the growth-promoting and anti-rachitic properties of the milk afforded by a cow. *Biochem J* **18**, 716-739.
17. Chick H & Roscoe MH (1926) Influence of diet and sunlight upon the amount of vitamin A and vitamin D in the milk afforded by a cow. *Biochem J* **20**, 632-649.
18. Thompson SY (1968) Section D. nutritive value of milk and milk products. Fat soluble vitamins in milk and milk products. *J Dairy Res* **35**, 149-169.
19. Thompson SY, Henry KM, Kon SK (1964) Factors affecting the concentration of vitamins in milk: I. Effect of breed, season and geographical location on fat-soluble vitamins. *J Dairy Res* **31**, 1-25.
20. Scott J, Bishop DR, Zechalko A *et al.* (1984) Nutrient content of liquid milk. 1. Vitamins A, D<sub>3</sub>, C and the B complex in pasteurised bulk liquid milk. *J Dairy Res* **51**, 37-50.
21. Kurmann KA & Indyk H. (1994) The endogenous vitamin D content of bovine milk: Influence of season. *Food Chem*, **50**, 75–81.
22. Lindmark-Månsson H, Fondén R, Pettersson HE (2003) Composition of Swedish dairy milk. *Int Dairy J* **13**, 409-425.
23. McCance RA & Widdowson E (2002) In *McCance and Widdowson's The Composition of Foods*, 6<sup>th</sup> Edition. Cambridge: Royal Society of Chemistry.
24. McCance RA & Widdowson E (2014) In *McCance and Widdowson's The Composition of Foods*, 7<sup>th</sup> Edition. Cambridge: Royal Society of Chemistry.
25. Hymøller L, Mikkelsen LK, Jensen SK *et al.* (2008) Access to outside areas during March and April in Denmark has negligible effect on the vitamin D<sub>3</sub> status of organic dairy cows. *Acta Agric Scand A* **58**, 51-54.

26. Bartlett S, Cotton AG, Henry KM *et al.* (1938) 196. The influence of various fodder supplements on the production and the nutritive value of winter milk. *J Dairy Res* **9**, 273-309.
27. European Commission (2016) European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Appendix 4(II). Annex II: List of additives subject to the provisions of Art. 10 2 of Reg. (EC) No 1831/2003 for which no application for revaluation was submitted before the deadline of 8 November 2010. Released 18<sup>th</sup> April 2016.
28. European Commission (2015) European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Released 14<sup>th</sup> December 2015.
29. Rode LM, McAllister TA, Cheng KJ (1990) Microbial degradation of vitamin A in rumen fluid from steers fed concentrate, hay or straw diets. *Can J Anim Science* **70**, 227–233.
30. Bourne N, Wathes DC, McGowan M *et al.* (2007) A comparison of the effects of parenteral and oral administration of supplementary vitamin E on plasma vitamin E concentrations in dairy cows at different stages of lactation. *Livest Sci* **106**, 57-64.
31. Horst RL, Reinhardt TA, Reddy GS (2005) Vitamin D metabolism. *Vitamin D* **1**, 15-36.
32. Thompson SY & Hidioglou M (1983) Effects of large oral and intravenous doses of vitamin D<sub>2</sub> and D<sub>3</sub> on the vitamin D in milk. *J Dairy Sci* **66**, 1638-1643.
33. Olsen WG, Jorgensen NA, Bringe AN *et al.* (1974) 25-Hydroxyvitamin D<sub>3</sub> (25-OH-D<sub>3</sub>). III. Effect of dosage on soft tissue integrity and vitamin D activity of tissue and milk from dairy cows. *J Dairy Sci* **57**, 677-682.
34. Weiss WP, Azem E, Steinberg W *et al.* (2015) Effect of feeding 25-hydroxyvitamin D<sub>3</sub> with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves. *J Dairy Sci* **98**, 5588-5600.
35. McDermott CM, Beitz DC, Littledike ET *et al.* (1985) Effects of dietary vitamin D<sub>3</sub> on concentrations of vitamin D and its metabolites in blood plasma and milk of dairy cows. *J Dairy Sci* **68**, 1959-1967.
36. Ramhola HMM, Santos J, Casal S *et al.* (2012) Fat-soluble vitamin (A, D, E and  $\beta$ -carotene) contents from the Portuguese autochthonous cow breed – Minhota. *J Dairy Sci* **95**, 5476-5484.
37. Department for Environment, Food and Rural Affairs (2008) The Cattle Book 2008: Descriptive statistics of cattle numbers in Great Britain). Released 1<sup>st</sup> June 2008.

- 378 38. Wallis GC (1944) A breed comparison in the vitamin D content of milk with notes on a  
 379 modified technique for the vitamin D assay of low-potency fats and oils. *J Dairy Sci* **27**, 733-  
 380 742.
- 381 39. Bechtel HE & Hoppert CA (1936) A Study of the Seasonal Variation of Vitamin D in Normal  
 382 Cow's Milk. *J Nutr* **11**, 537-49.
- 383 40. Pires P, Fernandes É, Vilarinho M *et al.* (2003) Comparison of milk from two different cow  
 384 breeds Barrosã and Frísia. *Electron J Environ Agric Food Chem* **2**, 514-518.
- 385 41. Hymøller L & Jensen SK (2010) Vitamin D<sub>3</sub> synthesis in the entire skin surface of dairy cows  
 386 despite hair coverage. *J Dairy Sci* **93**, 2025–2029.
- 387 42. Hymøller L & Jensen SK (2012) 25-Hydroxycholecalciferol status in plasma is linearly  
 388 correlated to daily summer pasture time in cattle at 56°N. *Br J Nutr* **108**, 666–671.
- 389 43. Libon F, Cavalier E, Nikkels AF (2013) Skin color is relevant to vitamin D synthesis.  
 390 *Dermatology* **227**, 250-254.
- 391 44. Wilkens MR, Cohrs I, Lifschitz AL *et al.* (2013) Is the metabolism of 25-hydrovitamin D<sub>3</sub>  
 392 age-dependent in dairy cows? *J Steroid Biochem Mole Biol* **136**, 44 – 46.
- 393 45. Okano T, Yokoshima K, Kobayashi T (1984) High-performance liquid chromatographic  
 394 determination of vitamin D<sub>3</sub> in bovine colostrum, early and later milk. *J Nutr Sci*  
 395 *Vitaminol* **30**, 431-439.
- 396 46. Kelsey JA, Corl BA, Collier RJ *et al.* (2003) The effect of breed, parity, and stage of lactation  
 397 on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J Dairy Sci* **86**, 2588-297.
- 398 47. Bainbridge ML, Cersosimo LM, Wright ADG *et al.* (2016) Content and composition of  
 399 branched-chain fatty acids in bovine milk are affected by lactation stage and breed of dairy  
 400 cow. *PLoS One* **11**, e0150386



401 **Table 1:** Studies investigating the seasonal variation of vitamin D concentrations in cow's milk

Study	Country	Cattle Breed	Study Design		Vitamin D Concentrations				
			<i>n</i>	Collection Date	Milk sample collected	<i>n</i>	Summer content	Winter content	<i>P</i>
Bechtel & Hoppert, 1936 <sup>(39)</sup>	United States of America	Guernsey; Holstein	8 cows; 14 cows	July 1932 - July 1934	Fresh milk	Monthly	Max - 0.78µg/quart; Max - 0.42µg/quart	Max - 0.12µg/quart; Max - 0.08µg/quart	Not reported
Thompson <i>et al.</i> , 1964 <sup>(2419)</sup>	England	Aryshire; Jersey; Friesian	60 cows; 60 cows; 50 cows	Early March 1960	Fresh milk - churned to butter for analysis	Single 15 gallon sample collected from each herd	0.016µg/g of fat 0.013µg/g of fat 0.011µg/g of fat	0.002µg/g of fat 0.002µg/g of fat 0.002µg/g of fat	Not reported
Scott <i>et al.</i> , 1984 <sup>(20)</sup>	Great Britain	Non-Channel Island breeds	12 dairies	May 1980 - September 1981	Pasturised milk	Every 7 weeks	0.033µg/100g of milk	0.026µg/100g of milk	Not reported
Kurmann & Indky, 1994 <sup>(21)</sup>	New Zealand	Friesian and Jersey-cross herds	1 processing site	August 1991 - May 1992	Bulk tank milk	Monthly	0.006µg/g of fat	0.002µg/g of fat	Not reported
Lindmark-Månsson <i>et al.</i> , 2003 <sup>(22)</sup>	Sweden	Not specified	9 dairies	November 1995 - November 1996	Bulk tank milk	Every 2 months	Range 0.01 - 0.12µg/100g* 0.03µg/100g	Mean	<0.001

\*Seasonal means not reported

402  
403 **Table 2:** Studies investigating the impact of cattle breed on the vitamin D concentrations of milk

Study Design						
Study	Country	Cattle Breed	<i>n</i>	Milk sample collected	<i>n</i>	Vitamin D Concentrations
Bechtel & Hoppert, 1936 <sup>(39)</sup>	United States of America	Guernsey; Holstein	8 cows; 14 cows	Fresh milk	Monthly; 1932 - 1934	0.12 - 1.09µg/quart; 0.08 - 0.69µg/quart
Wallis, 1944 <sup>(38)</sup>	United States of America	Jersey; Holstein	3 cows; 3 cows	Fresh milk - extracted butterfat	Monthly - for up to 13 months	0.75µg/quart; 0.25µg/quart
Thompson <i>et al.</i> , 1964 <sup>(19)</sup>	England	Aryshire; Jersey; Friesian	60 cows; 60 cows; 50 cows	Fresh milk - churned to butter for analysis	Single 15 gallon sample collected from each herd in early March	0.016µg/g of fat*; 0.013µg/g of fat*; 0.011µg/g of fat*
Pires <i>et al.</i> , 2003 <sup>(40)</sup>	Portugal	Barrosã; Friesian	5 cows; 5 cows	Fresh milk	1 from each cow	2.60µg/100g; 1.21µg/100g
Ramhola <i>et al.</i> , 2012 <sup>(36)</sup>	Portugal	Minhota; Holestein-Friesian	15 cows; 15 cows	Fresh milk	Monthly: October 2008 - September 2009	0.11µg/g of fat†; 0.10µg/g of fat†
McCance & Widdowson, 2014 <sup>(24)</sup>	United Kingdom	Jersey; UK pooled milk (breed not specified)	Not specified	Whole milk; whole, semi-skimmed and skimmed	6 (3 summer and winter); not specified	0.01µg/100g; 'trace' for whole, semi-skimmed and skimmed

\*Summer vitamin D concentration. Winter concentrations for all three breeds were 0.002µg/g of fat

†Provitamin D<sub>3</sub> also measured - mean concentrations higher in the milk of Holstein-Friesian than Minhota cattle (0.77µg and 0.45µg per gram of fat)

**Table 3:** Studies investigating the impact of hair coverage and dominant hair colour on the vitamin D synthesis

Study	Country	Cattle Breed	<i>n</i>	Study Duration	Study Design		Plasma 25(OH)D Concentrations		
					Treatment Groups	<i>n</i>	Mean at Baseline	Mean at End-point	<i>P</i>
Hymøller and Jensen, 2010 <sup>(41)</sup>	Denmark	Holstein	16 cows	28 days	Allocated to coverage;	14	2.5 ±0.2ng/ml	8.9±1.8ng/ml;	≤0.01
					Horse blanket;			23.2±1.5ng/ml;	
					Udder cover;			6.0±0.5ng/ml;	
					Horse blanket and udder cover;			28.6±3.1ng/ml	
Hymøller and Jensen, 2012 <sup>(42)</sup>	Denmark	Holstein	20 cows	28 days	Natural	14	44.9±2.4nmol/l	36.2±6.4nmol/l	≤0.001*
					Allocated to daily pasture for;			26.7±2.8nmol/l;	
					15mins;			43.9±8.5nmol/l;	
					30 mins;			67.4±8.6nmol/l;	
					75 mins;			95.9±6.4nmol/l	
					150 mins;				
					300 mins				

25(OH)D; 25-hydroxyvitamin D

\*While no figures were reported it was also determined that dominant hair colour (black or white) had no impact on plasma 25(H)D concentrations